He who dares only wins sometimes: physiological stress and contest behaviour in Xiphophorus helleri

He who dares only wins sometimes: physiological stress and contest behaviour in *Xiphophorus helleri*

Boulton K[1], Sinderman B[2], Pearce MR[2], Earley RL[2*] & Wilson AJ[1*]

[1] Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Ashworth Laboratories, King's Buildings, West Mains Road, Edinburgh, EH9 3JT, UK

* Department of Biological Sciences, University of Alabama, 300 Hackberry Lane, Box 870344, SEC Building, Tuscaloosa, AL 35487

[*] **Authors made equal contributions**

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Corresponding author: k.boulton@sms.ed.ac.uk

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SUMMARY

While many factors influence contest outcome and social dominance in animals, there is increasing interest in behavioural-physiological stress-coping styles. Causality, however, is often ambiguous – is physiological state determined by contest outcome or *vice versa*? Furthermore, experimental protocols may themselves induce stress responses that impact individual behaviour and thus potentially contest outcome. Here we test whether latency to recover from acute stress, measured both physiologically and behaviourally, predicts who initiates and who wins dyadic contests between pairs of male green swordtails *(Xiphophorus helleri)*. In line with our predictions, animals that recovered faster (behaviourally) from disturbance created by the experimental protocol prior to meeting an opponent were more likely to initiate contests; however, they were not more likely to win and, contrary to expectations, had higher pre-contest cortisol levels than their opponents. They also showed greater physiological stress responses to the experiment as determined from the difference between pre- and post-contest cortisol levels. Moreover, stress response was independent of whether a contest escalated. In contradiction to evidence found in other taxa and fish systems, the suite of traits that we measured were not correlated in a manner that allowed classification of the animals into the usual reactive and proactive stress-coping styles. Our results suggest that coping style may play a key role in determining the individual initiates a contest, but that other factors govern contest outcome.

INTRODUCTION

Competition for resources such as food, mates or territory, often involves contests where winners, or dominant individuals, improve their fitness at the expense of losers

40 (Brockelman, 1975). Many factors are expected to influence contest outcome and so
41 determine dominance status. While these are known to include size (e.g. Huntingford et al.,
42 1990) and behavioural traits such as aggression (Francis, 1988), individual styles of coping
43 with stress may also be important (Koolhaas et al., 1999; Pottinger & Carrick, 2001; Øverli et
44 al., 2004). Stress threatens homeostasis that is re-established by both physiological and
45 behavioural responses. Importantly, when studying behaviour, experimental protocols may
46 induce stress responses that impact individual behaviour, thus indirectly influencing
47 eventual contest outcome. Here we explore the hypothesis that latency to recover from
48 stress, as measured both behaviourally and physiologically, is a key determinant of contest
49 initiation and outcome. In animals, physiological stress-coping mechanisms are highly
50 conserved and governed by the hypothalamic-pituitary-adrenal (HPA) axis; in fish, this role is
51 assumed by the hypothalamic-pituitary-interrenal (HPI) axis, a good physiological indicator
52 being water-borne cortisol (for a review, see Scott & Ellis, 2007; Scott et al., 2008).

53 Classically, much research on animal conflict has focused on the concept of resource
54 holding potential (RHP; Parker, 1974). Commonly used measures of RHP (e.g. body size)
55 often predict contest initiation and outcome, although resource ownership, individual
56 motivation and social processes such as eavesdropping and prior fighting experience are
57 also important (Hsu et al., 2006; Arnott & Elwood, 2008). Studies that attempt to control
58 for RHP, for example by size matching and using neutral arenas, have suggested that
59 individuals initiating contests tend to win them (Jackson, 1991). However, this is not always
60 the case (Moretz, 2003), suggesting that factors other than the initial motivation to fight
61 may affect contest outcome especially during escalated contests (Hsu & Wolf, 2001).

62 The relationship between physiological stress (HPA/HPI axis activity) and social
63 dominance has received increasing attention and has been well studied across many taxa,
64 including rodents (Bronson, 1973), primates (Abbott et al., 2003), birds (Verbeek et al.,
65 1996), mammals (Young et al., 2006), domestic livestock (Bergsma et al., 2008) and fish
66 (Øverli et al., 2007). However, causality is often ambiguous; it is unclear whether
67 physiological state is determined by outcome, or outcome is determined by physiological
68 state. For example, faster recovery of baseline cortisol levels following aggressive contests
69 is associated with dominance (Netherton et al., 2004), while individuals with higher baseline
70 cortisol levels are less likely to win contests or to obtain dominance status in a hierarchy
71 (Hannes, 1984; DiBattista et al., 2005). Other types of behavioural variation may be linked
72 to physiological stress, particularly an individual's coping style (Earley et al., 2006). In a
73 study focusing on both behavioural and neuroendocrinological parameters, Koolhaas et al.
74 (1999) contrasted proactive and reactive coping styles and suggested a proactive/boldness
75 link (boldness is here described as a willingness to explore novel environments, Budaev,
76 1997). Many studies have demonstrated correlations between boldness and aggression (for
77 example, Bell & Sih, 2007), and of specific interest is that in fishes, empirical measurements
78 of HPI activity, aggression and boldness have been associated with differences in coping
79 style (Schjolden et al., 2005; Aubin-Horth et al., 2012).

80 The majority of studies investigating contest behaviour and dominance in domestic
81 and wild fishes use experimental designs that require netting individuals to facilitate periods
82 of isolation. This is usually followed by some form of disturbance, such as the removal of
83 partitions between isolated contestants in novel environments (for example, Wilson et al.,
84 2011a). Could it be that contest winners are those that better cope with stressors imposed

by the experimental protocol prior to even encountering an opponent? If so, then aspects of personality (e.g. boldness) and/or stress coping style may predict observed aggression and contest outcome.

Here we test the effect of disturbances imposed by the experimental protocol on contest behaviour and outcome using male green swordtails (*Xiphophorus helleri*), a small, tropical freshwater, live-bearing fish. Due to their readily aggressive nature, species from the *Xiphophorus* genus are commonly used as behavioural models in studies of dominance and many such studies have focused on visual and social cues as explanations for conflict resolution (Earley, 2006). However, we hypothesize that if coping style is important in the determination of observed contest behaviour under experimental situations, then relationships should exist between the behavioural reaction to disturbance prior to meeting an opponent, the likelihood of initiating a contest, contest outcome and the physiological stress response as measured by cortisol levels. Specifically, we predict that a short latency to resume normal swimming behaviour following disturbance will be associated with fish that initiate and win contests; such animals are predicted to be less stressed, i.e., have lower baseline (pre-contest) cortisol levels and a smaller stress response (post contest minus pre-contest cortisol level) than the eventual losers.

METHODS

Green swordtails (*Xiphophorus helleri*) obtained from a commercial distributor were housed in heterosexual groups in 152 and 208 L aquaria equipped with gravel substrate (3cm), filtration, and aeration. Water temperature was maintained between 23 - 25° C, pH between 7.2-7.6, and fish were kept on a 12 h light: 12 h dark photoperiod. Stress Coat™ (94µl/L) and freshwater aquarium salt (2g/L) were added to the tanks prior to fish arrival to

mitigate the loss of fish mucus and to reduce osmotic stress, respectively; each of these is a common response of fish to shipping and handling.

Dyad Establishment

Males were netted from the aquarium and placed in a plastic bag with a small amount of water to keep the gills and body moist and to immobilize the fish for measurement; measurements were taken with Vernier calipers accurate to 0.1 mm. Measurements of standard length (SL, snout tip to caudal peduncle), total body length (snout tip to caudal fin tip), body depth (BD, anterior portion of dorsal fin to origin of gonopodium), and sword length (SwL, caudal fin tip to sword tip) were obtained. Pairs of males for dyadic trials were matched for lateral surface area (LSA; < 20 units difference) because LSA has been shown to be a better predictor of fighting ability than any one measure of size alone (Beaugrand et al., 1996). LSA (mm^2) was determined as:

(standard length * body depth) + (sword length * sword depth)

assuming a sword depth of 1.0 mm. Body markings and coloration were also noted for purposes of identification. Macromelanophore patterns and sword characteristics were used to discriminate the two opponents (Franck et al., 2001; Basolo & Trainor, 2002). A total of 30 pairs were formed.

Contests and Hormone Collection

Immediately after measurements, fish were transferred directly from the plastic bag to 1000 ml polypropylene holding beakers containing 1000 ml of aerated freshwater. Stress Coat™ (94µl) and freshwater aquarium salt (2 g) were added to the holding container to replace fish mucus and reduce osmotic stress associated with handling during

130 measurement. The holding beakers were outfitted with a fine mesh net bottom and placed
131 inside another 1000 ml polypropylene beaker; this design made it possible to transfer the
132 fish between beakers gently, quickly (< 5 seconds) and without the handling typically
133 associated with capture (e.g., chasing, netting). The fish remained in the holding beaker for
134 2d to acclimate before being transferred to new 1000 ml sampling beakers containing 1000
135 ml of freshwater (with 4g freshwater salt) for 2 h, with hormones were released into the
136 water during this time (Scott et al., 2008). Stress Coat™ was not added to the hormone
137 collection beaker because it is not known whether the chemical interferes with hormone
138 extraction and assay; freshwater salt, however, can be purged from hormone extraction
139 columns (see below). After 2 h in the pre-fight sampling beaker the fish were transferred
140 using a net to 38 L experimental fight tanks, separated into two equal compartments by an
141 opaque divider. Each compartment was equipped with an aeration device and the water
142 was treated with Stress Coat™ and freshwater aquarium salt. The two fighters were placed
143 on opposite sides of the same fight tank and acclimated for 22 h. After this time the
144 dividers were lifted (remotely) and the air stones were also removed. This physical
145 disturbance typically resulted in frantic swimming behaviour by both fish, characterized by
146 fast, erratic movements both horizontally and vertically before the fish settled to the gravel
147 bottom. We therefore consider it to be a response imposed by the experimental protocol
148 itself. We determined the latency of behavioural recovery from this event as the time (from
149 lifting of partition) to resume normal swimming, defined as swimming slowly in a horizontal
150 orientation with fins often erect or semi-erect.

151 The fish then interacted until a dominance relationship was established, defined as
152 the point when one individual retreated 10 consecutive times without reciprocating

153 aggression or displayed typical submissive posturing, such as folding fins upon approach
154 from the opponent (Franck & Ribowski, 1989; Beaugrand, 1997). Contests lasted for an
155 average of 2286± 441 seconds and were recorded digitally using a Sony PC110 Digital Video
156 camera then burned to DVD. The identity of the animal that first began swimming normally
157 following partition removal, initiated the contest (approached within one body length of the
158 opponent), and won the contest was recorded using JWatcher version 1.0 (Blumstein &
159 Daniel, 2007; http://www.jwatcher.ucla.edu/). Latency to contest initiation, as well as
160 contest duration (from initiation to settlement) was calculated in seconds from partition
161 lifting. Additionally, we classified each contest as being escalated or not. Escalated contests
162 were defined as those involving high intensity reciprocal attacks, where the opponents
163 would alternate attack-bite sequences often while circling one another, and/or
164 mouthwrestling, where contestants would lock jaws in an apparent test of strength.
165 Immediately after contest resolution, fighters were netted and placed in individual 1000 ml
166 sampling beakers for 2 h for a post-fight hormone collection.

167 *Hormone Extraction and Radioimmunoassay*

168 C18 SPE columns (Extract-Clean®, 500 mg, 4.0 ml; Alltech Associates, Inc.) were
169 primed with 2 x 2 ml of 100% ethanol (EtOH) and 2 x 2 ml distilled water. Tygon tubing
170 (formulation 2275) was attached to the C18 columns and placed in a beaker containing a
171 250 ml water sample taken from the original 1000 ml, the vacuum was engaged and water-
172 borne steroid hormones isolated. Total hormone (free and conjugated fractions) was eluted
173 from the columns with 2 x 2 ml 100% ethanol collected in 6 ml (12 x 75 mm) borosilicate
174 vials. Samples were stored at 4° C overnight and the ethanol was evaporated in a Savant
175 AES 1010 speedvac for 1.5 h (45 min at 40ºC) one day prior to radioimmunoassay. Hormone

residues were resuspended in 60µl of 0.1 M phosphate buffer. Cortisol radioimmunoassay was conducted using a coat-a-count kit purchased through Diagnostic Products Corporation (Los Angeles, CA). Samples were run in duplicate in three separate assays conducted on three consecutive days. Briefly, 25 µl of each sample was pipetted into antibody-coated polypropylene tubes followed by the addition of 1 ml of I^{125}-labeled cortisol. Samples were incubated in a 37°C water bath for 45 min. Liquid in all samples was then decanted, and the tubes were blotted and allowed to air dry for 30 min prior to quantification. The sensitivities of the three assays were 0.0268 µg/dl, 0.033 µg/dl, and 0.0624 µg/dl. Pooled low-, medium- and high-level human serum (CON6 Multivalent Control Module, Diagnostic Products Corporation) were used as intra-assay controls; intra-assay coefficients of variation (assay 1, 2, and 3) were: tri-level low (6.2%, 3.8%, 2.1%), tri-level medium (2.8%, 12.0%, 4.3%), and tri-level high (4.8%, 5.0%, 7.2%). Inter-assay coefficients of variation were 6.4%, 7.5%, and 7.3% for tri-level low, tri-level medium and tri-level high, respectively.

The kit was validated for *X. helleri* by assessing parallelism and by calculating expected versus observed cortisol concentrations from known samples cold-spiked with standards. Twenty non-experimental swordtails (males and females) were transferred to collection beakers filled with 400 ml freshwater for 8h (0800-1600 h). Hormones were extracted and processed as described above, except that they were resuspended in 120µl and combined to form a pool of 2.4 ml stored as 55µl aliquots at –80 ºC. 240 µl of the pooled control was used for serial dilutions. Briefly, 120 µl of this sample was transferred to a 1.5 ml Eppendorf tube and mixed by vortexing with 120µl of 0.1 M phosphate buffer to create a 1:2 dilution; 120µl of 1:2 dilution was mixed with an equal volume of 0.1 M phosphate buffer to create a 1:4 dilution, and so on until 1:16. The serial dilutions were run

in quadruplicate using the RIA protocol described above with the Cortisol Coat-a-Count kit from DPC. The log-logit transformed dilution curve was parallel to the standard curve (comparison of slopes: $t_7 < 0.01$, $p > 0.05$; (Zar, 1996), p. 355). A 385µl sample of pooled hormone extract was used to assess recovery. 110µl was pipetted into a tube to constitute the 'neat' (1:1) control. 55µl of the large sample was then pipetted into 5 additional tubes and mixed with an equal volume of each standard provided with the DPC Cortisol coat-a-count kit (1, 5, 10, 20, 50 µg/dl). Expected recovery concentrations were based on the known amount of cortisol in the *X. helleri* control sample. Minimum recovery was 90.3% and the slope of the observed vs. expected curve was 0.97, demonstrating a highly linear relationship between observed and expected recovery.

One fish died during the period of post-contest cortisol collection and therefore data relating to the trial that it participated were excluded from analysis. A further pair was eliminated because they did not interact on any level. A total of 28 contests from the original 30 pairs of fish were therefore observed, where 25 produced clear winners and losers and 15 were classified as escalated. The first individual to swim normally following partition removal and the individual that initiated the contest was unambiguously determined in all 28 cases (see supplemental material Table S1 for raw data on all contests).

Data analysis

In order to summarize associations among the full set of morphological, behavioural, and endocrine traits measured we generated a correlation matrix using Genstat 14.1 (Payne et al., 2005). Correlations between morphological and physiological traits were estimated using the full set of observations (i.e. one record per individual, n=56) for body depth (BD), standard length (SL), sword length (SwL), lateral surface area (LSA), pre-contest (PreCORT)

and post-contest (PostCORT) cortisol levels and physiological stress response (SR). Endocrine assays before and after the trial were $\log_{10}$ transformed to yield PreCORT and PostCORT respectively, while we defined SR as the change in cortisol expression on a $\log_{10}$ scale (i.e., SR= PostCORT – PreCORT). For those traits where the phenotypic value of one individual within a trial necessarily determines that of the second, we used observations from one randomly chosen focal individual per trial only (n = 28). These traits include the binary variables of Swimfirst (whether the focal fish was first to resume normal swimming after disturbance), Initiate (whether the focal fish initiated the contest) and Status (whether the focal fish was the winner). For these randomly chosen focal individuals we also determined a relative measure of size difference (LSAdiff), defined as the difference in phenotypic values (focal LSA – opponent LSA). Correlations with two further traits, latency to swim (LatSwim) and latency to initiate (LatInit) were also estimated. However, these traits are only meaningfully observed for the individual within each trial that either swims first or initiates the contest, respectively. Thus estimated correlations with these variables are conditional on moving first or initiating the contest as appropriate (n=28).

To more directly test the hypothesized causal relationships between behavioural recovery from disturbance, contest initiation and outcome (i.e., status) and stress response, we formulated a set of linear models that were solved by restricted maximum likelihood using ASReml (Version 3, Gilmour et al., 2009). In particular this allowed us to test our hypotheses while properly accounting for any influence of body size (LSA) on endocrine traits and/or contest behaviour. Note therefore that our phenotypic measures of the endocrine traits (PreCORT, PostCORT, SR) are not corrected in any way for the expected

influence of fish size (Scott & Ellis, 2007) prior to analysis; rather, the linear model framework allows us to control for these effects statistically within the analysis.

As described above, each contest provides only a single phenotypic observation for the binary traits of Initiate (Model 1) and Status (Model 2) and these response variables were analysed using generalized linear models (with logit link function). Thus we modelled probability (on the logit scale) of initiating a contest as a function of being first to adjust to normal swimming behaviour following removal of the partition (*Swimfirst*), as well as baseline cortisol (*PreCORT*), size (*LSA*), and all two-way interactions of these explanatory variables such that:

$$Initiate_{ik} = \mu + Swimfirst + PreCORT + LSA + Swimfirst.PreCORT + Swimfirst.LSA + PreCORT.LSA + \varepsilon_k \quad \text{(Model 1)}$$

Where $Initiate_{ik}$ is the probability (on the logit scale) of individual *i* initiating contest *k*, μ is an overall mean, and ε is a residual error term (assumed to be uncorrelated across trials). The probability of winning a contest (*Status*, 0/1) was modelled in a similar way, but with the addition of fight *Escalation* (as a two-level categorical variable, i.e. whether a fight did or did not escalate) fitted as a factor, and its interaction terms as additional explanatory effects. Escalation is included here because Swimfirst may only predict contest winners when fights do not escalate (e.g., see Hsu & Wolf, 2001).

$$Status_{ik} = \mu + Swimfirst + PreCORT + Escalation + LSA + Swimfirst.PreCORT + Swimfirst.LSA + PreCORT.Escalation + PreCORT.LSA + Escalation.LSA + \varepsilon_k \quad \text{(Model 2)}$$

Finally we modelled stress response (*SR*) to test the hypothesis that it would be lower for those individuals that had won contests, and particularly so in the absence of contest escalation. Values of SR can be assigned to both individuals within a trial but may not be fully independent. We therefore analysed SR using a linear mixed effect model (with normal error structure) that included a random effect of trial to account for non-independence (Model 3).

$$SR_{ik} = \mu + Swimfirst + Status + Escalation + LSA + Swimfirst.LSA + Swimfirst.Status + Swimfirst.Escalation + Status.LSA + Status.Escalation + Escalation.LSA + Trial_k + \varepsilon_k \quad \text{(Model 3)}$$

For each of the models shown above we adopted a model reduction strategy where explanatory terms were dropped if they were statistically non-significant at $p \geq 0.1$ under a two-tailed conditional *F* - test. Main effects were retained in the model if one or more of their interactions were retained on this basis. Note that we chose to use a threshold of α=0.1 rather than 0.05 in our model reduction strategy and therefore our final models can contain marginally non-significant explanatory terms (i.e. $0.1 \leq p \leq 0.05$). We adopted this strategy as, since available sample sizes are fairly small we expect power will be limiting. However, we deem that it is instructive to consider whether marginally non-significant terms are at least qualitatively consistent with hypothesized biological processes, i.e. it may not be sensible to equate non-significance with an effect size of zero.

RESULTS

Among trait correlations

The estimated correlation structure provided evidence of significant associations among a number of the traits measured (Table 1). Phenotypic correlations were close to unity among the morphological traits of BD, SL and LSA ($r_{BD.SL} = 0.95$, $r_{BD.LSA} = 0.99$, $r_{SL.LSA} = 0.98$; *all* $p<0.001$), an unsurprising result given that these all capture aspects of body size. Sword length (SwL) was also positively correlated with body size traits although less strongly. Body size traits were significantly and positively correlated with both pre- and post-contest cortisol levels (r ranging from 0.42 - 0.48, all $p \leq 0.001$; Table 1) although again the correlation between PostCORT and sword length (SwL) was lower($r = 0.30$, $p = 0.03$). Given that endocrine traits are not standardised for size variation prior to analysis these results are consistent with the expectation of a positive association between body size and cortisol release into the water (Scott et al., 2008), controlled for in our model based hypothesis testing (as discussed above). Note that stress response (SR) is auto-correlated with pre- and post-contest cortisol levels as a consequence of its definition (i.e. SR = PreCORT – PostCORT; $r_{SR.PreCORT} = - 0.43$, and $r_{SR.PostCORT} = 0.43$, both $p =0.001$). Cortisol levels before and after the contest were also significantly correlated within individuals ($r_{PreCORT, PostCORT} = 0.64$, p = <0.001). However, correlations between SR and size (as measured by the various morphology traits) are weak and non-significant.

Among behavioural traits we found a significant positive correlation between swimming first and initiating the contest as we hypothesized ($r = 0.56$, $p = 0.004$). For the set of individuals that both swam first and initiated the contest, latency to swim was also strongly correlated with latency to initiate ($r = 0.64$, $p = 0.003$). However, swimming first was not positively correlated with status (i.e. winning, $r = -0.16$, $p = 0.58$), and among those fish that did swim first the correlation between latency to swim and status was close to zero

310 (r = 0.16, p = 0.45). Thus the correlation structure is consistent with our hypothesis that
311 individuals more rapidly resuming normal swimming after partition removal are more likely
312 to initiate contests. However, these individuals are not more likely to win the subsequent
313 contest.

314 The correlation structure provided only limited statistical support for relationships between
315 behavioural and endocrine traits. Contrary to our expectation that individuals exhibiting
316 lower baseline cortisol, i.e., presumably less stressed prior to the trial, would move first, we
317 actually found a positive, albeit weak and non-significant, correlation between preCORT and
318 Swimfirst (r = 0.006, p = 0.98). Higher PreCORT was significantly associated with an
319 increased tendency to initiate the contest (r = 0.45, p = 0.025). Both PreCORT and PostCORT
320 levels were negatively correlated with latency to swim (among fish that swam first) and the
321 relationship was significant in both cases ($r_{PreCORT.LatSwim} = -0.45$, $p = 0.024$, $r_{PostCORT.LatSwim} = -0.70$, $p < 0.001$). Negative correlations of similar magnitude were found between PreCORT
322
323 and PostCORT and the latency to initiate a contest; however, only the PostCORT correlation
324 was significant ($r_{PreCORT.LatInit} = -0.39$, $p = 0.10$, $r_{PostCORT.LatInit} = -0.47$, $p = 0.04$) (Table 1).

325 *Model based hypothesis testing*

326 Model 1 supported our hypothesis that individuals that swim first would also initiate
327 contests more often (p = 0.029); however, contrary to our *a priori* expectation that contest
328 initiators would have lower levels of pre-contest cortisol, higher PreCORT levels were in fact
329 associated with contest initiators (p = 0.036, Table 2). These patterns are qualitatively
330 consistent with the significant correlation structure among initiate, PreCORT and Swimfirst
331 as reported above. The estimated effect of PreCORT on tendency to initiate was more
332 convincing in the reduced model (3.03 ± 1.37 µg/dl) than in the full model (-7.34 ± 15.64

μg/dl). This could reflect the fact that the latter estimate of the PreCORT effect is conditioned on the putative dependence on body size (although neither LSA nor its interactions were statistically significant). Model 2 provided no evidence that contest winning is predicted by swimming first or by baseline physiological stress (i.e. PreCORT). These findings are counter to our second *a priori* hypothesis, but again consistent with the simple correlation analysis. Although we also tested for dependency of these effects on contest escalation and/or size effects, in fact no explanatory variables were retained in the reduced version of Model 2. Thus we were unable to predict contest outcome from size, behaviour, or baseline physiological stress. Finally, although stress response was lower in contest winners as we had predicted, the difference between losers and winners was not significant in the full model (-0.40 ± 0.46 μg/dl, $p = 0.90$) and therefore status was not retained in our reduced model (Model 3). However, based on a marginally non-significant interaction of Swimfirst and size (LSA) ($p = 0.071$, Table 2) both variables were retained in the reduced model. Under the full model for stress response, 5 (± 23) % of the observed variance not explained by fixed effects was explained by Trial. Under the reduced model, the corresponding estimate was 14 (± 19) % of the variance. The random effect of trial is not significant in either the full ($p = 0.83$) or the reduced ($p = 0.49$) models.

DISCUSSION

The primary goals of this study were to determine firstly whether the latency to recover behaviourally from an acutely stressful event commonly employed in behavioural experiments – lifting partitions - could explain variation in contest behaviour and outcome. Secondly, we wanted to test whether this latency was related to endocrine measures of physiological stress obtained from water-borne cortisol assays. Our first prediction was that

356 fish more rapidly resuming normal swimming behaviour following removal of a partition in a
357 dyadic behavioural trial would tend to initiate and win contests. These relationships among
358 behavioural traits were not supported by our data, suggesting that a proactive coping style
359 is associated with readjusting to experimental protocol disturbances; however, it is not
360 associated with initiating or winning contests. Although many studies on fish have found a
361 strong positive association between initiating and winning contests (e.g., Jackson, 1991; Hsu
362 et al., 2009), our data suggest that we should be careful in assuming this pattern will always
363 hold.

364 Both the correlation analysis and the linear models, where potentially confounding
365 effects of body size could be statistically accounted for (Scott & Ellis, 2007), revealed some
366 associations between behavioural and endocrine traits. However, these associations were
367 not consistent with our *a priori* predictions. For example, we predicted that behavioural
368 recovery following a partition being lifted would be faster for fishes with lower baseline
369 (pre-contest) cortisol levels; however, the reverse pattern was seen. While this effect was
370 non-significant, pre-contest cortisol level was significantly and positively associated with
371 tendency to initiate contests. Pre-contest cortisol level did not predict contest outcome,
372 and there was no significant effect of status on stress response. Although SR was lower in
373 winners as we predicted the effect size was small and non-significant.

374 Overall our results do not fit comfortably into the proactive-reactive framework that
375 has been used to interpret suites of correlated traits as reported in mammalian, avian, and
376 other fish systems (Koolhaas et al., 1999; Øverli et al., 2007; Carere et al., 2010). Some
377 recent studies provide evidence consistent with this framework, testing the hypothesis that
378 differences in behaviour are associated with differences in stress response (Øverli et al.,

2002; Øverli et al., 2005; Øverli et al., 2007). These studies found that those individuals more rapidly resuming normal behaviour in novel environments or following acute stress were socially more dominant and in addition, had lower baseline cortisol levels and stress-responsive cortisol levels than those taking longer to resume normal behaviour. Thus, individuals have been argued to lie along a continuum of coping styles ranging from proactive to reactive, respectively. It should be noted that these fish studies were carried out using lines of domestic rainbow trout (*Oncorhynchus mykiss*) specifically selected for divergent cortisol responses; however, more recent work focussing on variation within populations has reached similar conclusions in a range of wild and domestic fish species (see Conrad et al., 2011 for a comprehensive review).

The swordtails used for our study were captive bred and, although they had wild-type colours, have an unknown history of artificial selection under conditions of high resource availability with environmental stressors likely to differ substantially from those of wild fish. We certainly acknowledge that relaxed natural selection in captivity might result in increased phenotypic variance and/or behaviour-physiology correlations that are either unexpected or that would be maladaptive in the wild (e.g., Lee & Berejikian, 2008; Conrad & Sih, 2009). We also acknowledge that our sample size was relatively small, thus limiting statistical power, and that control experiments to examine physiological responses to barrier removal without a subsequent dyadic contest would be useful. Nevertheless, it is equally true that other studies conducted under both laboratory and field conditions have reported deviations from the expected trait correlation structure among proactive – reactive coping style extremes, suggesting that the categorization is too simplistic (Brelin et al., 2008; Archard & Braithwaite, 2011; Vaz-Serrano et al., 2011; Archard et al., 2012).

Environmental context can dissolve or generate trait correlations (e.g, Bell & Sih, 2007), and even completely reverse relationships between behaviour and physiology (Ruiz-Gomez et al. 2008). These studies suggest considerable plasticity in trait associations and the involvement of multiple, perhaps independently operating mechanisms that shape associations between behaviour and endocrine state.

Evidence from studies of behaviour in male tree lizard morphs (Thaker et al., 2009) suggests that animals with elevated cortisol levels are more prepared for an immediate response to predators. Koolhaas et al., (1997) suggested that elevations of glucocorticoids at appropriate times can be adaptive, in that they prepare the animal for immediate environmental unpredictability. Speculatively, it is possible that in our study we have uncovered a similar finding: animals with already elevated cortisol levels recover more quickly from stressors and therefore behave, at least initially, in a proactive manner. Similarly, contest winners may simply be reacting more quickly on a physiological level both to the disturbance from the experimental protocol and the attack from the proactive opponent. If this were indeed the case then a higher overall stress response for the reactive individual would seem to be appropriate.

Variation in endocrine traits did not match all our *a priori* expectations. *Post hoc* analyses revealed significant variance among-individuals that may have important functional consequences. Specifically, a *post hoc* mixed model analysis showed that after conditioning on size (LSA) and sampling point (i.e., pre- or post-trial) $\log_{10}$ transformed cortisol levels were repeatable (interclass correlation of 0.26 (± 0.13), $\chi^2_{1DF} = 6.16$, P = 0.013). This highlights the fact that there is among-individual variation (and within individual consistency) in assayed cortisol levels, beyond that attributable to size variation). This

model also confirmed the expected increase in cortisol levels with LSA (0.002 (± 0.0004), $F_{1,54DF} = 11.38$, $p = 0.002$), and also that average cortisol levels were higher post-trial (difference of 0.125 ((± 0.046) on the $\log_{10}$ scale , $F_{1,55DF} = 7.52$, $p = 0.008$) consistent with a positive physiological reaction, i.e., stress response, to the contest and/or experimental protocol. However, there was variation in SR and indeed 18 of 56 fish actually had lower cortisol release rates (i.e., SR<0) in response to barrier removal and social challenge.

Furthermore, neither the causes nor the consequences of this among-individual variance are known at present. Such differences could emerge if individuals experience size- and status-dependent shifts in gill permeability to steroid hormones (e.g., Scott et al., 2008), i.e., a change in stress responsive release rates reflects the ability of steroids to leak across the gills for water-borne hormone measurement. Alternatively, given the inherent lag between spikes in plasma and water-borne hormones, we could be observing the confluence of status- and size-dependent differences in within-contest cortisol production. Acute elevations of stress hormone have been associated with increased aggression during social interactions (e.g., Kruk et al., 2004; Earley et al., 2006). Although we do not know if the association between acute stress responses, aggression, and social dominance is size-dependent, it is possible that large winners mounted a stronger within-contest stress response than is detectable in the water-borne sample.

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REFERENCES

Abbott, D. H., Keverne, E. B., Bercovitch, F. B., Shively, C. A., Medoza, S. P., Saltzman, W., Snowdon, C. T., Ziegler, T. E., Banjevic, M., Garland, T. & Sapolsky, R. M. (2003). Are subordinates always stressed? A comparative analysis of rank differences in cortisol levels among primates. — Hormones and Behavior 43, 67-82.

Archard, G. A. & Braithwaite, V. A. (2011). Increased exposure to predators increases both exploration and activity level in Brachyrhaphis episcopi. — Journal of Fish Biology 78, 593-601.

Archard, G. A., Earley, R. L., Hanninen, A. F. & Braithwaite, V. A. (2012). Correlated behaviour and stress physiology in fish exposed to different levels of predation pressure. — Functional Ecology 26, 637-645.

Arnott, G. & Elwood, R. W. (2008). Information gathering and decision making about resource value in animal contests. — Animal Behaviour 76, 529-542.

Aubin-Horth, N., Deschênes, M. & Clouter, S. (2012). Natural variation in the molecular stress network correlates with a behavioural syndrome. . — Hormones and Behavior **61**, 140-146.

Basolo, A. L. & Trainor, B. C. (2002). The conformation of a female preference for a composite male trait in green swordtails. — Animal Behaviour 63, 469-474.

Beaugrand, J. P. (1997). Resolution of agonistic conflicts in dyads of acquainted green swordtails (Xiphophorus helleri): a game with perfect information (vol 41, pg 79, 1997). — Behavioural Processes 41, 293-310.

Beaugrand, J. P., Payette, D. & Goulet, C. (1996). Conflict outcome in male green swordtail fish dyads (Xiphophorus helleri): Interaction of body size, prior dominance/subordination experience, and prior residency. — Behaviour 133, 303-319.

Bell, A. & Sih, A. (2007). Exposure to predation generates personality in threespined sticklebacks (Gasterosteus aculeatus). — Ecology Letters 10, 828-834.

Bergsma, R., Kanis, E., Knol, E. F. & Bijma, P. (2008). The Contribution of Social Effects to Heritable Variation in Finishing Traits of Domestic Pigs (Sus scrofa). — Genetics 178, 1559-1570.

Blumstein, D. T. & Daniel, J. C. (2007). Quantifying Behavior the JWatcher Way —Sinauer Associates, Inc. .

Brelin, D., Petersson, E., Dannewitz, J., Dahl, J. & Winberg, S. (2008). Frequency distribution of coping strategies in four populations of brown trout (Salmo trutta). — Hormones and Behavior 53, 546-556.

Brockelman, W. Y. (1975). Competition, fitness of offspring and optimal clutch size. — American Naturalist 109, 677-699.

Bronson, F. H. (1973). Establishment of social rank among grouped male mice - relative effects on circulating FSH, LH and corticosterone. — Physiology & Behavior 10, 947-951.

Budaev, S. V. (1997). "Personality" in the guppy (Poecilia reticulata): A correlational study of exploratory behavior and social tendency. — Journal of Comparative Psychology 111, 399-411.

Carere, C., Caramaschi, D. & Fawcett, T. W. (2010). Covariation between personalities and individual differences in coping with stress: Converging evidence and hypotheses. — Current Zoology 56, 728-740.

Conrad, J. L. & Sih, A. (2009). Behavioural type in newly emerged steelhead Oncorhynchus mykiss does not predict growth rate in a conventional hatchery rearing environment. — Journal of Fish Biology 75, 1410-1426.

Conrad, J. L., Weinersmith, K. L., Brodin, T., Saltz, J. B. & Sih, A. (2011). Behavioural syndromes in fishes: a review with implications for ecology and fisheries management. — Journal of Fish Biology 78, 395-435.

DiBattista, J. D., Anisman, H., Whitehead, M. & Gilmour, K. M. (2005). The effects of cortisol administration on social status and brain monoaminergic activity in rainbow trout Oncorhynchus mykiss. — Journal of Experimental Biology 208, 2707-2718.

Earley, R. (2006). *Xiphophorus*: Carving a Niche Towards a Broader Understanding of Aggression and Dominance. — Zebrafish 3, 283-293.

Earley, R. L., Edwards, J. T., Aseem, O., Felton, K., Blumer, L. S., Karom, M. & Grober, M. S. (2006). Social interactions tune aggression and stress responsiveness in a territorial cichlid fish (Archocentrus nigrofasciatus). — Physiology & Behavior 88, 353-363.

Francis, R. C. (1988). On the relationship between aggression and social dominance. — Ethology 78, 223-237.

Franck, D., Dikomey, M. & Schartl, M. (2001). Selection and the maintenance of a colour pattern polymorphism in the green swordtail (Xiphophorus helleri). — Behaviour 138, 467-486.

Franck, D. & Ribowski, A. (1989). Escalating fights for rank-order position between male swordtails *(Xiphophorus helleri) :* Effects of prior rank-order experience and information transfer*. — Behavioral Ecology and Sociobiology 24, 133-143.

Gilmour, A. R., Gogel, B. J., Cullis, B. R. & Thompson, R. (2009). ASReml user guide release 3.0. — VSNi, Hemel Hempstead, UK.

Hannes, R. P. (1984). Fighting changes levels of androgens and corticoids in winner and loser swordtails differently. — Aggressive Behavior 10, 156-156.

Hsu, Y., Lee, I. H. & Lu, C.-K. (2009). Prior contest information: mechanisms underlying winner and loser effects. — Behavioral Ecology and Sociobiology 63, 1247-1257.

Hsu, Y. Y., Earley, R. L. & Wolf, L. L. (2006). Modulation of aggressive behaviour by fighting experience: mechanisms and contest outcomes. — Biological Reviews 81, 33-74.

Hsu, Y. Y. & Wolf, L. L. (2001). The winner and loser effect: What fighting behaviours are influenced? — Animal Behaviour 61, 777-786.

Huntingford, F. A., Metcalfe, N. B., Thorpe, J. E., Graham, W. D. & Adams, C. E. (1990). Social dominance and body size in Atlantic salmon parr, Salmo salar L. — Journal of Fish Biology 36, 877-881.

Jackson, W. M. (1991). Why Do Winners Keep Winning? — Behavioral Ecology and Sociobiology 28, 271-276.

Koolhaas, J. M., deBoer, S. F. & Bohus, B. (1997). Motivational systems or motivational states: Behavioural and physiological evidence. — Applied Animal Behaviour Science 53, 131-143.

Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., De Jong, I. C., Ruis, M. A. W. & Blokhuis, H. J. (1999). Coping styles in animals: current status in behavior and stress-physiology. — Neuroscience and Biobehavioral Reviews 23, 925-935.

Kruk, M. R., Halasz, J., Meelis, W. & Haller, J. (2004). Fast positive feedback between the adrenocortical stress response and a brain mechanism involved in aggressive behavior. — Behavioral Neuroscience 118, 1062-1070.

Lee, J. S. F. & Berejikian, B. A. (2008). Effects of the rearing environment on average behaviour and behavioural variation in steelhead. — Journal of Fish Biology 72, 1736-1749.

Moretz, J. A. (2003). Aggression and RHP in the northern swordtail fish, Xiphophorus cortezi: The relationship between size and contest dynamics in male-male competition. — Ethology 109, 995-1008.

Netherton, J. D., Grober, M. S. & Earley, R. L. (2004). Temporal decay of cortisol in green swordtail fish (Xiphophorus helleri) following aggressive encounters: Differences between winners and losers? — Hormones and Behavior 46, 117-118.

Øverli, O., Korzan, W. J., Hoglund, E., Winberg, S., Bollig, H., Watt, M., Forster, G. L., Barton, B. A., Øverli, E., Renner, K. J. & Summers, C. H. (2004). Stress coping style predicts aggression and social dominance in rainbow trout. — Hormones and Behavior 45, 235-241.

Øverli, O., Pottinger, T. G., Carrick, T. R., Øverli, E. & Winberg, S. (2002). Differences in behaviour between rainbow trout selected for high- and low-stress responsiveness. — Journal of Experimental Biology 205, 391-395.

Øverli, O., Sorensen, C., Pulman, K. G. T., Pottinger, T. G., Korzan, W. J., Summers, C. H. & Nilsson, G. E. (2007). Evolutionary background for stress-coping styles: Relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates. — Neuroscience and Biobehavioral Reviews 31, 396-412.

554 Øverli, O., Winberg, S. & Pottinger, T. G. (2005). Behavioral and neuroendocrine correlates of
555 selection for stress responsiveness in rainbow trout - a review. — Integrative and
556 Comparative Biology 45, 463-474.
557 Parker, G. A. (1974). Assessment strategy and evolution of fighting behavior. — Journal of
558 Theoretical Biology 47, 223-243.
559 Payne, R. W., Murray, D. M., Harding, S. A., Baird, D. B. & Soutar, D. M. (2005). GenStat for
560 Windows, Introduction. — VSN International Ltd. , Hemel Hempstead, UK.
561 Pottinger, T. G. & Carrick, T. R. (2001). Stress responsiveness affects dominant-subordinate
562 relationships in rainbow trout. — Hormones and Behavior 40, 419-427.
563 Schjolden, J., Stoskhus, A. & Winberg, S. (2005). Does individual variation in stress responses
564 and agonistic behavior reflect divergent stress coping strategies in juvenile rainbow
565 trout? — Physiological and Biochemical Zoology 78, 715-723.
566 Scott, A. P. & Ellis, T. (2007). Measurement of fish steroids in water - a review. — General and
567 Comparative Endocrinology 153, 392-400.
568 Scott, A. P., Hirschenhauser, K., Bender, N., Oliveira, R., Earley, R. L., Sebire, M., Ellis, T., Pavlidis,
569 M., Hubbard, P. C., Huertas, M. & Canario, A. (2008). Non-invasive measurement of
570 steroids in fish-holding water: important considerations when applying the procedure
571 to behaviour studies. — Behaviour 145, 1307-1328.
572 Thaker, M., Lima, S. L. & Hews, D. K. (2009). Acute corticosterone elevation enhances
573 antipredator behaviors in male tree lizard morphs. — Hormones and Behavior 56, 51-
574 57.
575 Vaz-Serrano, J., Ruiz-Gomez, M. L., Gjoen, H. M., Skov, P. V., Huntingford, F. A., Øverli, O. &
576 Hoglund, E. (2011). Consistent boldness behaviour in early emerging fry of
577 domesticated Atlantic salmon (Salmo salar): Decoupling of behavioural and
578 physiological traits of the proactive stress coping style. — Physiology & Behavior 103,
579 359-364.
580 Verbeek, M. E. M., Boon, A. & Drent, P. J. (1996). Exploration, aggressive behavior and
581 dominance in pair-wise confrontations of juvenile male great tits. — Behaviour 133,
582 945-963.
583 Wilson, A. J., Boer, M. d., Arnott, G. & A.Grimmer. (2011). Integrating personality research and
584 animal contest theory: aggressivemness in green swordtail *Xiphophorus helleri*. — Plos
585 One *6(11)*
586 Young, A. J., Carlson, A. A., Monfort, S. L., Russell, A. F., Bennett, N. C. & Clutton-Brock, T. (2006).
587 Stress and the suppression of subordinate reproduction in cooperatively breeding
588 meerkats. — Proceedings of the National Academy of Sciences of the United States of
589 America 103, 12005-12010.
590 Zar, J. H. (1996). *Biostatistical analysis*. — Prentice-Hall, Upper Saddle River, NJ.
591
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Table 1.

Phenotypic trait correlation matrix. The full data set was used to estimate correlations between the morphology and physiology traits of body depth (BD), standard length (SL), sword length (SwL), lateral surface area (LSA), Pre- (PreCORT) and post-contest (PostCORT) cortisol levels, and stress response (SR). The randomly selected half data set was used to calculate correlations between traits with only one phenotypic observation per trial: Status, Swimfirst, Initiate and differences in lateral surface area between opponents in the same contest (LSAdiff). Correlations for the traits latency to swim (LatSwim) and latency to initiate (LatInit) are calculated using one observation per trial, conditional on swimming first or initiating the contest. Bold font denotes a significant correlation (2-tailed $p<0.05$). Bold italic font denotes a marginally non-significant correlation (2-tailed $p<0.1$).

	BD	**SL**	**SwL**	**LSA**	**Pre CORT**	**Post CORT**	**SR**	**Status**	**Swim first**	**Initiate**	**LSA diff**	**Lat Swim**	**Lat Init**
BD	-												
SL	**0.949**	-											
SwL	**0.308**	**0.429**	-										
LSA	**0.987**	**0.984**	**0.391**	-									
PreCORT	**0.432**	**0.477**	**0.418**	**0.453**	-								
PostCORT	**0.422**	**0.434**	**0.296**	**0.425**	**0.639**	-							
SR	-0.013	-0.050	-0.144	-0.033	**-0.425**	**0.425**	-						
Status	0.021	0.044	-0.200	0.037	-0.157	-0.191	-0.009	-					
Swimfirst	0.040	0.014	0.001	0.040	0.006	0.273	0.315	-0.116	-				
Initiate	0.016	0.059	0.172	0.042	**0.449**	***0.370***	-0.181	-0.131	**0.559**	-			
LSAdiff	0.104	0.164	-0.164	0.128	-0.181	-0.133	0.092	***0.344***	0.016	-0.202	-		
LatSwim	-0.163	-0.204	***-0.382***	-0.187	**-0.450**	**-0.695**	-0.086	0.157	*	-0.178	***0.344***	-	
LatInit	-0.228	-0.321	**-0.464**	-0.286	***-0.392***	**-0.474**	-0.035	0.359	*	*	***0.412***	**0.642**	-

*Correlation not available

Table 2. ANOVA table of fixed effects fitted in full and reduced linear models of Initiate, Status and Stress Response. Indicated are estimated effect sizes for explanatory terms fitted (with SE in parentheses), and conditional F tests. Initiate and status are modelled as binary response variables while a normal error structure was fitted for stress response. Where used as explanatory variables Swimfirst, Status and EscalF were fitted as two level factors with the estimated coefficients denoting the effect of factor level 1 (fish swam first, fish won the contest, contest was escalated) relative to factor level 0. Models of stress response also included a random effect of trial (see text for details).

		FULL MODEL				REDUCED MODEL			
Trait	**Fixed Effect**	**Coefficient (SE)**	**DF**	**F**	**p**	**Coefficient (SE)**	**DF**	**F**	**p**
Initiate	mu	-2.37 (8.28)	1,21	0.59	0.449	-1.52 (0.802)	1,25	0.04	0.838
	Swimfirst	9.35 (9.92)	1,21	3.71	0.068	2.48 (1.07)	1,25	5.35	0.029
	PreCORT	-7.38 (15.6)	1,21	4.61	0.044	3.03 (1.37)	1,25	4.90	0.036
	LSA	0.001 (0.019)	1,21	1.49	0.235				
	PreCORT.LSA	0.033 (0.036)	1,21	0.84	0.368				
	Swimfirst.LSA	-0.015 (0.023)	1,21	0.42	0.522				
	Swimfirst.PreCORT	-0.781 (6.50)	1,21	0.01	0.906				
Status	mu	-7.33 (9.19)	1,14	0.84	0.375	-0.080 (0.400)	1,24	0.04	0.843
	Swimfirst	4.27 (8.90)	1,14	0.08	0.778				
	PreCORT	-2.42 (7.21)	1,14	1.43	0.252				
	EscalF	2.32 (7.66)	1,14	0.06	0.804				
	LSA	0.018 (0.021)	1,14	0.86	0.371				
	Swimfirst.PreCORT	3.72 (3.94)	1,14	0.89	0.361				
	Swimfirst.EscalF	1.68 (2.07)	1,14	0.66	0.431				
	Swimfirst.LSA	-0.013 (0.021)	1,14	0.38	0.548				
	PreCORT.LSA	-0.007 (0.014)	1,14	0.25	0.625				
	PreCORT.EscalF	1.91 (3.18)	1,14	0.36	0.557				
	EscalF.LSA	-0.007 (0.018)	1,14	0.15	0.707				
Stress Response	mu	0.094 (0.455)	1,39	1.99	0.167	-0.220 (0.295)	1,26.2	0.62	0.438
	Swimfirst	0.824 (0.456)	1,19	0.13	0.720	0.758 (0.383)	1,26	0.42	0.525
	Status	-0.402 (0.456)	1,19	0.02	0.903				

EscalF	-0.467 (0.500)	1,20	0.42	0.522				
LSA	-0.002 (0.001)	1,20.1	0.02	0.885	0.001 (0.001)	1,26.2	0.05	0.824
Swimfirst.Status	0.038 (0.211)	1,20	0.03	0.858				
Swimfirst.EscalF	-0.081 (0.202)	1,19	0.16	0.694				
Swimfirst.LSA	-0.002 (0.001)	1,19.1	3.31	0.085	-0.002 (0.001)	1,26.1	3.56	0.071
Status.EscalF	-0.120 (0.202)	1,19	0.35	0.559				
Status.LSA	0.001 (0.001)	1,19.1	1.10	0.307				
EscalF.LSA	0.001 (0.001)	1,20.1	1.15	0.295				

Supplemental material.

Table S1

Detail of morphological and physiological measurements for individual fish, where: Trial is the competition that the individual participated in; Fish is the identity assumed in the particular trial; SL is Standard Length; BD is Body Depth; SwL is sword length; LSA is Lateral Surface Area; Lat Swim is latency to swim; Lat Init is latency to initiate; Status is W, win and L, lose; PreCORT is pre-contest cortisol level; PostCORT is Post-contest cortisol level; SR is stress response; Escal denotes fight escalation (Y) per trial.

Trial	Fish	SL (mm)	BD (mm)	SwL (mm)	LSA (mm2)	Lat Swim (secs)	Lat Init secs)	Status	Pre CORT (µg/dl)	Post CORT (µg/dl)	SR	Escal
1	A	46.50	14.00	23.40	674.40	10	63	L	3.800	1.223	-2.576	N
1	B	45.05	14.80	13.00	679.74	*	*	W	0.809	2.962	2.153	-
2	A	43.60	13.20	17.30	592.82	*	*	W	0.895	5.065	4.171	Y
2	B	43.80	13.30	13.40	595.94	67	98	L	2.258	3.807	1.549	-
4	A	40.10	11.90	12.70	489.89	7	11	L	3.049	2.630	-0.419	Y
4	B	39.80	12.20	11.90	497.46	*	*	W	1.044	1.440	0.396	-
5	A	35.05	11.00	14.20	399.75	3	*	L	1.337	4.554	3.218	Y
5	B	35.65	10.70	13.30	394.76	*	14	W	1.227	4.717	3.491	-
7	A	37.20	10.65	18.20	414.38	*	*	L	1.242	1.826	0.585	Y
7	B	37.20	10.75	17.75	417.65	103	109	W	3.350	1.695	-1.655	-
8	A	35.10	10.30	16.05	377.58	6	24	W	2.110	1.634	-0.476	Y
8	B	35.15	10.00	15.65	367.15	*	*	L	1.663	2.281	0.619	-
9	A	42.20	12.05	24.70	533.21	85	92	W	2.909	2.322	-0.587	Y
9	B	42.50	12.20	18.25	536.75	*	*	L	1.450	2.046	0.596	-
10	A	39.95	11.75	20.30	489.71	*	*	L	1.534	1.840	0.306	Y
10	B	40.35	12.10	18.50	506.74	51	196	W	1.315	2.394	1.078	-
11	A	48.80	13.90	19.00	697.32	242	*	W	0.889	1.369	0.480	N
11	B	46.60	14.15	20.55	679.94	*	254	L	3.292	4.259	0.967	-
12	A	32.60	9.25	9.30	310.85	127	184	W	0.159	0.805	0.646	Y
12	B	33.85	9.30	7.95	322.76	*	*	L	0.740	0.986	0.246	-
13	A	38.40	11.50	21.85	463.45	*	*	W	1.623	1.338	-0.285	Y
13	B	39.20	11.50	20.20	471.00	14	26	L	1.958	4.684	2.726	-
14	A	37.50	10.80	16.00	421.00	*	*	L	1.337	1.820	0.483	Y
14	B	37.40	10.90	17.60	425.26	240	465	W	1.514	0.673	-0.841	-
15	A	41.40	12.25	17.60	524.75	246	*	*	0.593	0.256	-0.337	N
15	B	40.10	12.35	17.50	512.74	*	304	*	1.060	0.321	-0.739	-
16	A	39.70	11.20	15.00	459.64	*	181	L	1.371	1.030	-0.340	N
16	B	38.70	11.30	15.10	452.41	101	*	W	0.380	0.889	0.508	-
17	A	34.85	10.00	12.05	360.55	66	1275	W	0.952	1.104	0.151	N
17	B	35.60	10.00	11.75	367.75	*	*	L	1.868	3.177	1.308	-
18	A	33.90	9.90	9.35	344.96	*	*	W	0.638	0.516	-0.122	Y
18	B	33.30	10.00	13.45	346.45	640	641	L	0.617	0.163	-0.454	-
19	A	39.85	10.90	20.05	454.42	*	1640	*	1.258	1.102	-0.156	Y
19	B	39.75	11.15	22.60	465.81	116	*	*	0.567	2.120	1.554	-
20	A	35.80	10.10	22.35	383.93	109	155	L	0.742	2.279	1.537	N
20	B	36.75	10.00	22.40	389.90	*	*	W	0.977	1.246	0.269	-
21	A	33.60	9.60	15.15	337.71	*	*	W	0.350	0.241	-0.108	N
21	B	33.30	9.25	16.90	324.93	27	30	L	0.205	0.892	0.687	-
22	A	35.60	10.30	14.50	381.18	278	*	L	0.102	0.327	0.224	N
22	B	34.60	10.40	13.70	373.54	*	354	W	1.132	1.151	0.019	-
23	A	41.90	12.30	11.00	526.37	*	*	W	0.578	0.596	0.018	Y

23	B	41.90	12.35	13.45	530.92	421	460	L	1.621	2.267	0.646	-
24	A	39.50	11.25	14.60	458.98	17	121	L	0.722	2.704	1.981	Y
24	B	40.05	10.95	16.75	455.30	*	*	W	1.993	2.179	0.186	-
25	A	33.40	9.60	18.00	338.64	61	96	L	2.876	2.271	-0.606	N
25	B	35.00	9.80	17.30	360.30	*	*	W	0.536	0.744	0.207	-
26	A	39.20	10.40	19.50	427.18	46	48	*	2.499	1.700	-0.799	N
26	B	37.65	10.50	16.30	411.63	*	*	*	1.686	1.814	0.128	-
27	A	34.15	10.05	10.80	354.01	142	*	L	0.120	0.642	0.522	N
27	B	34.85	10.00	10.50	359.00	*	189	W	0.429	0.329	-0.100	-
28	A	35.00	9.50	13.40	345.90	*	*	W	0.300	0.303	0.003	N
28	B	33.40	9.40	11.60	325.56	83	108	L	1.120	0.516	-0.604	-
29	A	34.40	10.12	11.30	359.43	540	*	W	0.069	0.358	0.290	N
29	B	34.42	9.80	18.85	356.17	*	586	L	0.468	2.099	1.630	-
30	A	32.85	9.26	17.50	321.69	*	*	L	0.179	0.094	-0.085	Y
30	B	33.15	10.00	9.30	340.80	554	3202	W	0.308	0.412	0.104	-